Title: Transfection of murine and human hematopoietic progenitors with rearranged immunoglobulin genes

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Contract number: N00014-89-C0305

Reporting Period: October 1991 - February 1992.

Work Summary: There are three main objectives of the research conducted under the above contract. First to isolate hematopoietic stem cells in numbers adequate for transfection with rearranged immunoglobulin genes. Second, to develop techniques which will allow B cells expressing the transfected Ig-gene product to be activated by anti-idiotypic antibodies so that high levels of serum antibody are produced. Finally, to develop a human-mouse chimera that will allow us to transfect rearranged Ig-genes into human hematopoietic progenitors and then grow and activate those cells in an animal model.

Section 1: Characterization of Antibodies Derived from Electroporation of Phosphocholine-Binding Heavy and Light Chain Genes into Myeloma Target Cells

In the in vivo selection and activation of B-cells by anti-idiotypic antibodies, there exist the possibility of stimulating B-cells which will express antibodies bearing cross-reactive idiotopes but these antibodies may not have specificity for the desired target antigen. Thus, activation of such cells will not result in production of protective levels of the desired antigen-specific antibody in the serum of the treated animal. In an attempt to understand the molecular requirements for phosphocholine-binding and idiotype expression, we have produce a group of cell lines by electroporation of heavy and light chain genes from know PC-binding myelomas and hybridomas into a non-productive myeloma cell line. The resulting antibodies were analyzed for their ability to bind PC and for expression of various idiotype markers. The clear existence of antibodies that bear idiotopes associated with PC-specificity but which fail to bind phosphocholine (PC)-BSA were obtained in cell lines 2,4,7,8 and 10 (Table 1). In several cases, such as cell line 1 vs 4, cell line 2 vs 5, and cell 9 vs 3, single amino acid changes in the heavy chain cause the loss in ability to bind antigen. The loss of antigen binding may result in loss of an individual idiotope as in cell line 4 where the insertion of an alanine in the H-chain sequence of cell line 1 causes both the loss of PC binding and the loss of the AB1.2 idiotype. Surprisingly, when one compares cell lines 2,5 and 11, it is only cell line 11 which exhibits good affinity for PC-BSA but all three lines express the binding-site-specific 28-5-15 idiotope. Thus, when one attempts to do in vivo activation using the 28-5-15 anti-idiotypic antibody, B-cells bearing these 3 types of antibodies would be stimulated even though we would only want the antibody expressed in cell line 11, which is the H:L gene combination that we are trying to put into the stem cells. It is now important to perform the in vivo experiments and determine how much of a problem the potentially cross-reactive endogenous antibodies will cause.

Section 1a: Isolation and characterization of early hematopoietic progenitor cells

As described in the annual report, we have been isolating early murine hematopoietic progenitor cells based on the early stem cell antigen, SCA-1. Previous studies have shown that in normal bone marrow, the percentage of SCA-1-positive stem cells is very low. However, as few as 30 of these cells can repopulate lethally irradiate animals and result in 50% survival 30 days after irradiation. Since the stem cell isolated from normal bone marrow is quiescent, there is a lag period required before expansion and repopulation of the animal. This lag period requires that intermediate, short-term hematopoiesis be initiated or the animal will die. Thus, it is customary to inject carrier bone marrow along with the stem cell to allow for survival of the animal during the time required for expansion of the stem cell. Our initial studies used putative stem cells isolated from B6D2F1 mice which were injected into SCID mice along with carrier BALB/c bone marrow. Our studies showed good engraftment of the MHC-matched BALB/c bone marrow with little successful competition by the MHC-mismatched B6D2F1 stem cell. One possibility for this selective advantage toward engraftment of the BALB/c bone marrow could be based on MHC compatibility. Studies to determine whether B6D2F1 bone marrow was equal to BALB/c bone marrow in the ability to engraft SCID mice were performed. Total bone marrow from both B6D2F1 and BALB/c mice were able to engraft equally well in the SCID mouse. Immunoglobulin production and mature B cells were observed in mice reconstituted with either 10^4 or 10^5 total bone marrow. However, if one removed mature, lineage-restricted cells from the bone marrow, the resulting lineage-negative populations (progenitor enriched population) from the two strains of mice exhibited different abilities to engraft SCID mice. Only the BALB/c lineage negative bone marrow reconstituted the B lymphoid arm of the immune system and resulted in the detection of serum Ig and mature B cells in the bone marrow and spleen of SCID mice. These preliminary data indicate that as one purifies progenitor cells, the ability to reconstitute MHC-mismatched recipients decreases. In this situation, it is not surprising that if present, MHC-matched carrier bone marrow would be favored over purified, MHC-mismatched bone marrow during reconstitution.

As stated previously, SCA-1-positive cells are normally quiescent. However, treatment of mice with 5-fluorouracil has been shown to increase the number and cycling status of primitive cells. We have exploited these finding in two ways: 1) use of 5-FU bone marrow due to the increased percentages of SCA-1-positive cells and 2) use of this regimen to hasten the engraftment of bone marrow cells in vivo. 5-FU bone marrow cells were harvested from B6D2F1 mice and cultured in vitro for 2 days with various cytokines. Following in vitro culture, cells were injected into lethally irradiated SCID mice in the absence of carrier bone marrow. Reconstitution was determined 3 months later based on the percentage of mature donor-derived B cells in the recipient bone marrow and spleen. Preliminary data show that injection of 10^4 cells cultured in either IL-3 alone or Stem Cell Factor (SCF) alone resulted in significant B cell engraftment in the bone marrow (9% and 20% respectively). The combination of both IL-3 and SCF did not result in detectable B cells in the bone marrow. In contrast, injection of 10^4 cells cultured in medium, IL-3, SCF, or IL-3/IL-

6 resulted in detectable mature B cells in the spleen of SCID animals. Furthermore, 10^3 cells cultured in IL-3/IL-6 also resulted in detectable B cells in the spleen. These data suggest that 5-FU bone marrow may not require carrier bone marrow to allow for long term reconstitution and that in vitro culture of this bone marrow in the presence of SCF or IL-3 may increase the engraftment and subsequent development of donor B cells in SCID mice. Further studies regarding the reconstitution of SCID mice using 5-FU-enriched, SCA-1-positive cells cultured with and without cytokines will be investigated. Alleviating the need for carrier bone marrow which may compete for the purified B6D2F1 stem cells would be advantageous.

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NWW 2/6/92

Table I.

Characterization of Cell Lines Electroporated with PC-Binding H and L Chain Genes[a)]

Cell Line	Transfected V-genes	Kappa	IgM[a]	V_H1-id	T15-id			M167-id		PC-BSA
					T139.2	AB1.2	PolyV	28-5-15	28-4-3	
1.	VT15μ Vκ22	+	+	+	+	+	+	-	-	+
2.	VT15μ V_κ24	+	+	+	-	-	-	+	+	-
3.	VT15μ V_κ8	+	+	+	-	-	-	-	-	+/-
4.	VT15μ-Ala V_κ22	+	+	+	+	-	+	-	-	-
5.	VT15μ-Ala V_κ24	+	+	+	-	-	-	+	+	+/-
6.	VT15μ-Ala V_κ8	+	+	+	-	-	-	-	-	-
7.	VT15μ-Asn V_κ22	+	+	+	+	+	+	-	-	-
8.	VT15μ-Asn V_κ24	+	+	+	-	-	-	-	+	-
9.	VT15μ-Asn V_κ8	+	+	+	-	-	-	-	-	+
10[b].	V167μ V_κ22	+	+	+	+	-	+	-	-	-
11.	V167μ V_κ24	+	+	+	-	-	-	+	+	+
12.	V167μ V_κ8	+	+	+	-	-	-	-	-	-

Footnotes Table 1

a) Cell lines designated with a + in a given assay produced an antibody which bound within a 2 fold range of the antibody used to generate the standard curve; +/-indicates that a quantity 20 to 40 fold lower than the standard curve antibody was obtained at the 1 μg/ml dilution; and a - means than nothing was detected at any dilution tested.